Diet-sensitive prognostic markers for cardiovascular and renal disease

Riphagen, Ineke Jowanna

Chapter 4

Copeptin, a Surrogate Marker for Arginine Vasopressin, is Associated with Cardiovascular and All-Cause Mortality in Patients with Type 2 Diabetes (ZODIAC-31)

Ineke J. Riphagen
Wendy E. Boertien
Alaa Alkhalaf
Nanne Kleefstra
Ron T. Gansevoort
Klaas H. Groenier
Kornelis J.J. van Hateren
Joachim Struck
Gerjan Navis
Henk J.G. Bilo
Stephan J.L. Bakker

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Abstract

Objective. Copeptin, a surrogate marker for vasopressin, has been associated with cardiovascular (CV) events and mortality in patients with type 2 diabetes complicated by end-stage renal disease or acute myocardial infarction. For stable outpatients, these associations are unknown. Our aim was to investigate whether copeptin is associated with CV and all-cause mortality in patients with type 2 diabetes treated in primary care.
Research Design and Methods. Patients with type 2 diabetes participating in the observational ZODIAC study were included. Cox regression analyses with age as time scale were used to assess the relation of baseline copeptin with CV and all-cause mortality.
Results. We included 1,195 patients (age 67 ± 12 years, 44% male). Median baseline copeptin concentration was 5.4 [IQR 3.1-9.6] pmol/L. After a median follow-up of 5.9 [3.2-10.1] years, 345 patients died (29%), with 148 CV deaths (12%). Log_2 copeptin was associated with CV (HR 1.17 [95% CI 0.99-1.39], *P*=0.068) and all-cause mortality (1.22 [1.09-1.36], *P*=0.001) after adjustment for age, gender, BMI, smoking, systolic blood pressure, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, ACEi/ARB, history of CV diseases, log serum creatinine, and log ACR. However, copeptin did not substantially improve risk prediction for CV (IDI 0.14 [-0.27-0.55]%) and all-cause mortality (IDI 0.77 [0.17-1.37]%) beyond currently used clinical markers.
Conclusions. We found copeptin to be associated with CV and all-cause mortality in patients with type 2 diabetes treated in primary care. Intervention studies should show whether the high CV risk in type 2 diabetes can be reduced by suppression of vasopressin, e.g. by reducing salt intake.

Introduction

The prevalence of type 2 diabetes and its complications are increasing worldwide (1). One of the major complications in type 2 diabetes is cardiovascular disease (CVD), which is the main cause of morbidity and mortality in this patient group (2).

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is a hormone exerting cardiovascular and renal effects (3). Several studies have reported that AVP levels are elevated in animals and patients with diabetes mellitus (4-7). Increased levels of AVP may have long-term deleterious effects. AVP acts through three different vasopressin receptors, namely the V_{1a}, V_2, and V_3 (or V_{1b}) receptors, which mediate vasoconstriction, stimulate water retention, and facilitate secretion of adrenocorticotropic hormone (ACTH) respectively (3). High concentrations of plasma AVP are known to preferably stimulate V_{1a} receptors (8), which may contribute to the cardiovascular complications in type 2 diabetes.

Despite the pivotal role of AVP in cardiovascular disease, technical difficulties due to AVP's small size, short plasma half-life time, and association of circulating AVP with platelets have hindered the large-scale clinical use of AVP as a biomarker (3,9,10). Vasopressin is synthesized via a polypeptide precursor which contains AVP, neurophysin II, and copeptin (3). Copeptin, or C-terminal proarginine vasopressin (CT-proAVP), is released in equimolar amounts to AVP during precursor processing and was found to be a stable and sensitive surrogate marker for AVP (11,12).

A recent study of Fenske et al. (8) showed that copeptin levels were strongly associated with cardiovascular events and mortality in patients with type 2 diabetes and end-stage renal disease (ESRD). Copeptin was also found to be associated with cardiovascular events in patients with acute myocardial infarction and type 2 diabetes (13).

However, to our knowledge, these associations have not been demonstrated for stable ambulatory patients with type 2 diabetes. This is of particular interest, since it would point to a new modifiable system for treatment and prevention of cardiovascular events and mortality in type 2 diabetes (8,14). Our primary objective was to assess the association of baseline plasma copeptin levels with cardiovascular and all-cause mortality in a population of patients with type 2 diabetes treated in primary care. Our secondary aim was to investigate the additional predictive value of copepin for risk prediction of cardiovascular and all-cause mortality in patients with type 2 diabetes.

Research Design and Methods

Study group

The Zwolle Outpatient Diabetes Project Integrating Available Care (ZODIAC) study was initiated in 1998, in the Zwolle region of the Netherlands. The design and details of this study have been published elsewhere (15,16). In this study, general practitioners were assisted by hospital-based diabetes specialist nurses in their care of patients with type 2 diabetes. In the first year, 1,143 patients with type 2 diabetes were included in this prospective cohort study. In 2001, 546 patients with type 2 diabetes enrolled in addition, leaving a combined cohort of 1,689 patients (17). Baseline plasma copeptin values were measured in 1,257 (74%) patients. In this study, we included 1,195 (95%) patients with complete data. The ZODIAC study was approved by the local medical ethics committee, and all patients provided informed consent.

Data collection and measurements

Baseline data were collected in 1998 and 2001, consisting of a full medical history including a history of cardiovascular diseases, use of medication, and tobacco consumption. Patients were considered to have a history of CVD if they had a history of angina pectoris, myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, stroke or transient ischemic attack. Laboratory and physical assessment data were collected annually and included non-fasting lipid profile, glycated hemoglobin (HbA_{1c}), serum creatinine (SCr), urinary albumin-to-creatinine ratio (ACR), and blood pressure. SCr was measured by a kinetic colorimetric Jaffe method (Modular P Analyzer, Roche Almere, the Netherlands), ACR was measured using immunonephelometry (Behring Nephelometer; Mannheim, Germany), and blood pressure was measured twice with a Welch Allyn sphygmomanometer in the supine position after at least 5 minutes of rest. The creatinine-based CKD-EPI equation was used to estimate glomerular filtration rate (eGFR) (18).

Copeptin was measured in plasma samples collected at baseline and kept frozen at -80° Celsius until analysis in 2010. Morgenthaler et al. (12) showed that prolonged frozen storage and repeated freeze-thaw cycles have no effect on copeptin values. Plasma copeptin was measured using a sandwich immunoassay (B.R.A.H.M.S. GmbH, Hennigsdorf/Berlin, Germany) based on the assay described by Morgenthaler et al. (12). Measurement of copeptin was performed in batches. The lower detection limit was 0.4 pmol/L, the interassay coefficient of variation (CV) was <6% for copeptin concentrations >6 pmol/L, and the functional assay sensitivitiy (20% interassay CV) was less than 1 pmol/L.

Clinical end points

In this study, we examined the association between baseline copeptin concentration and two co-primary clinical endpoints: cardiovascular mortality and all-cause mortality. In 2009, vital status and cause of death were retrieved from records maintained by the hospital and the general practitioners for the first 1,143 patients. For the additional 546 patients, vital status and cause of death were retrieved in 2005. Causes of death were coded according to the International Classification of Diseases, 9th revision (ICD-9). Cardiovascular death was defined as death in which the principal cause of death was cardiovascular in nature, using ICD-9 codes 390-459.

Statistical analyses

Statistical analyses were performed using SPSS version 18.0 for Windows (SPSS inc., Chicago, Illinois, USA) and STATA version 11 (StataCorp., College Station, TX: StataCorp LP). Results were expressed as mean ± standard deviation (SD) or median [interquartile range] for normally distributed and non-normally distributed data, respectively. Nominal data were presented as the number of patients (percentage). A two-sided *P*-value <0.05 was considered to indicate statistical significance.

For illustrative purposes, the study population was subdivided in tertiles of baseline copeptin concentration, and data were presented accordingly in order to visualize associations with copeptin. Since copeptin concentrations are significantly higher in men than in women (19), tertiles were gender-stratified. P-values for differences in copeptin tertiles were assessed using ANOVA for normally distributed continuous data, the Kruskal-Wallis test for non-normally distributed data, and the χ^2-test for nominal data. Multivariable linear regression analyses were used to investigate whether baseline copeptin concentrations were associated with clinical parameters. Since copeptin values were non-normally distributed, logarithmic transformation (base 2) was applied to fulfill the criteria for linear regression analyses.

We investigated whether there were differences in baseline characteristics of patients with and without copeptin measurement. *P*-values for differences between patients with and without copeptin measurement were assessed using the independent sample t-test for normally distributed continuous data, the Mann-Whitney U-test for non-normally distributed data, and the χ^2-test for nominal data.

Cox regression analyses were used to test whether there were interactions between copeptin and clinical parameters including age, gender, history of CVD, and duration of diabetes.

We used Cox regression analyses with age as the primary time scale in which we accounted for left truncation (delayed entry) to analyze the risk of cardiovascular and

all-cause mortality during follow-up. We applied $\log_2$ transformation of copeptin values so the hazard ratios derived from Cox regression analyses were expressed as an increase in risk per doubling of baseline copeptin values. Various models were built to adjust for possible confounders. First, the univariable association of $\log_2$ copeptin with cardiovascular and all-cause mortality was investigated. Second, the model was adjusted for age and sex. Finally, the model was additionally adjusted for cardiovascular risk factors and medication which could potentially influence copeptin secretion (i.e., BMI, smoking, systolic blood pressure, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, history of CVD, use of angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARB), log SCr, and log ACR). The assumption of proportional hazards for baseline predictors was investigated by inspecting the Schoenfeld residuals. As sensitivity analyses, we repeated the Cox regression analyses with follow-up time as time scale.

Furthermore, we investigated the effect of inclusion of time-dependent covariates (i.e., age, systolic blood pressure, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, SCr, and ACR) in Cox regression analyses.

In addition, Cox regression analyses were used to test whether an association existed between the presence or absence of a copeptin measurement and cardiovascular and all-cause mortality in the combined cohort of 1,689 patients.

Discrimination, a measure to evaluate how well a model distinguishes between patients who died and those who survived while taking follow-up time into account, was assessed using the Harrell's C statistic (20). The additional value of copeptin for the risk prediction of cardiovascular and all-cause mortality was assessed in terms of integrated discrimination improvement (IDI) and net reclassification improvement (NRI) (21). The IDI can be interpreted as the difference between model-based probabilities for events and non-events for the models with and without copeptin. The NRI is calculated by assessing the net improvement in risk classification (<10, 10-20, 20-30, and >30%) for events and non-events separately. Calibration, a measure to evaluate how well predicted probabilities agree with observed risks, was assessed using the Grønessby and Borgan 'goodness-of-fit' likelihood-ratio test (22).

Results

Patient characteristics

A total of 1,195 patients with type 2 diabetes were included in this study. Median age of the study population was 67 ± 12 years, and 524 patients (44%) were male. Median copeptin concentration was 5.4 [3.1-9.6] pmol/L. Copeptin concentrations were significantly higher in men than in women, 7.4 [4.5-11.5] versus 4.1 [2.6-7.3] pmol/L, *P*<0.001. Baseline patient characteristics are presented as gender-stratified tertiles in Table 1. Variables that were significantly different between tertiles of copeptin concentrations were age, history of CVD, BMI, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, ACR, SCr, and eGFR (Table 1). Multivariable linear regression analyses showed that gender (b = -0.57, *P*<0.001), age (b = 0.01, *P*<0.001), BMI (b = 0.03, *P*<0.001), HbA_{1c} (b = 0.11, *P*<0.001), systolic blood pressure (b = -0.004, *P*<0.001), log ACR (b = 0.21, *P*<0.001), and log SCr (b = 3.69, *P*<0.001) were associated with baseline copeptin concentrations.

In addition, we investigated whether there were differences in baseline patient characteristics of patients with and without copeptin measurement (Supplementary Table S1). Only baseline serum creatinine values were slightly higher in patients without copeptin measurement (98 ± 23 µmol/L) compared to patients with copeptin measurement (95 ± 22 µmol/L, *P*=0.04). We found no other significant differences between baseline characteristics of patients with and without copeptin measurement.

Copeptin as a predictor for mortality

After a follow-up period of 10 years for the patients entering the study in 1998 and 3 years for those included in 2001, 345 out of 1,195 included patients had died (29%), with 148 deaths (12%) attributable to cardiovascular causes. There was no larger number of deaths (152 males [29%] vs. 193 females [29%], *P*=0.9) or cardiovascular deaths (64 males [12%] vs. 84 females [13%], *P*=0.9) in male subjects compared to female subjects. Furthermore, there was no difference in follow-up time for male and female subjects (5.5 [3.1-10.1] years vs. 6.2 [3.2-10.1] years, respectively; *P*=0.4). The median copeptin level of patients who had died of cardiovascular causes (7.9 [4.1-12.9] pmol/L) as well as the median copeptin level of all patients who had died during follow-up (7.5 [3.7-12.6] pmol/L) were significantly higher than the median baseline copeptin concentration for survivors (4.9 [3.0-8.5] pmol/L), both *P*-values <0.001.

We found no significant interactions between copeptin and clinical parameters including age, gender, history of CVD, and duration of diabetes.

Table 1. Baseline patient characteristics of the study population presented as gender-stratified tertiles of copeptin concentration.

		Copeptin			
		Tertile 1	Tertile 2	Tertile 3	*P*-value
Copeptin (pmol/L)	M	<5.2	5.2-9.9	>9.9	-
	F	<3.1	3.1-5.9	>5.9	-
N	M	175	174	175	-
	F	224	227	219	-
Demographics					
Age (years)		65 ± 10	66 ± 12	69 ± 12	<0.001
Smoking (n, %)		79 (19.8)	73 (18.2)	61 (15.5)	0.3
History of CVD (n, %)		114 (28.5)	129 (32.2)	167 (42.4)	<0.001
Body composition					
BMI (kg/m^2)		28.5 ± 4.2	29.3 ± 4.8	29.9 ± 5.2	<0.001
Blood pressure					
Systolic blood pressure (mmHg)		153 ± 24	153 ± 25	151 ± 24	0.3
Diastolic blood pressure (mmHg)		84 ± 10	83 ± 10	84 ± 10	0.8
Use of ACEi/ARB (n, %)		102 (25.5)	102 (25.4)	117 (29.7)	0.3
Lipids					
Total cholesterol (mmol/L)		5.4 ± 1.0	5.6 ± 1.1	5.6 ± 1.1	0.1
HDL cholesterol (mmol/L)		1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.6	0.5
Triglycerides (mmol/L)		2.0 [1.5-2.8]	2.1 [1.5-3.1]	2.1 [1.5-3.2]	0.2
Cholesterol-HDL ratio		4.7 ± 1.5	5.0 ± 1.5	5.0 ± 1.4	0.04
Use of lipid lowering drugs (n, %)		61 (15.3)	62 (15.5)	50 (12.7)	0.5
Glucose homeostasis					
Duration diabetes (years)		4.0 [1.8-9.0]	4.0 [1.8-8.9]	5.0 [2.0-10.0]	0.002
HbA_{1c} (%)		6.8 [6.2-7.8]	7.0 [6.3-8.2]	7.3 [6.5-8.4]	<0.001
HbA_{1c} (mmol/mol)		51 [44-62]	53 [45-66]	56 [48-68]	<0.001
Renal function					
ACR (mg/mmol)		1.6 [0.9-4.0]	1.7 [0.8-6.1]	2.7 [1.0-8.2]	<0.001
Serum creatinine (µmol/L)		90 ± 14	91 ± 16	103 ± 29	<0.001
eGFR (mL/min/1.73m^2)		68 ± 14	67 ± 15	59 ± 18	<0.001

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; eGFR, estimated glomerular filtration rate; F, females; HDL, high-density lipoprotein; M, males.

Table 2. Associations of baseline log_2 copeptin concentrations with cardiovascular and all-cause mortality in Cox regression analyses with age as time scale.

	Cardiovascular Mortality		All-Cause Mortality	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Model 1	1.55 (1.34-1.80)	<0.001	1.39 (1.26-1.53)	<0.001
Model 2	1.51 (1.30-1.77)	<0.001	1.36 (1.22-1.51)	<0.001
Model 3	1.17 (0.99-1.39)	0.068	1.22 (1.09-1.36)	0.001

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; HDL, high-density lipoprotein; SCr, serum creatinine.
Model 1: crude.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, BMI, smoking, SBP, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, history of CVD, use of ACEi/ARB, log SCr, and log ACR.

In univariable Cox regression analyses with age as time scale, log_2 copeptin was significantly associated with cardiovascular mortality (HR 1.55 [95% CI 1.34-1.80], *P*<0.001) and all-cause mortality (1.39 [1.26-1.53], *P*<0.001). The association of copeptin with all-cause mortality remained significant after adjustment for the different confounders (Table 2). The Schoenfeld residuals showed no substantial deviations, supporting the assumption of proportional hazards.

As sensitivity analyses, we repeated the Cox regression analyses with follow-up time as time scale (Table 3). The hazard ratios and 95% confidence intervals of the adjusted models in the sensitivity analyses were not materially different from the analyses with age as time scale. In the Cox regression models with follow-up time as time scale, the associations of copeptin with cardiovascular and all-cause mortality remained significant after adjustment for the different confounders (Table 3).

We also investigated the influence of time-dependent covariates in Cox regression analyses (Supplementary Table S2). In the fully adjusted models, log_2 copeptin was significantly associated with cardiovascular mortality (1.21 [1.03-1.42], *P*=0.02) and all-cause mortality (1.23 [1.10-1.36], *P*<0.001).

Furthermore, we tested whether an association existed between the presence or absence of a copeptin measurement and cardiovascular and all-cause mortality in the combined cohort of 1,689 patients. In univariable and multivariable Cox regression analyses we found no association of the presence or absence of a copeptin measurement with cardiovascular and all-cause mortality.

Table 3. Associations of baseline log_2 copeptin concentrations with cardiovascular and all-cause mortality in Cox regression analyses with follow-up time as time scale.

	Cardiovascular Mortality		All-Cause Mortality	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Model 1	1.74 (1.49-2.04)	<0.001	1.57 (1.42-1.74)	<0.001
Model 2	1.54 (1.32-1.80)	<0.001	1.37 (1.24-1.52)	<0.001
Model 3	1.19 (1.00-1.41)	0.04	1.23 (1.10-1.37)	<0.001

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; HDL, high-density lipoprotein; SCr, serum creatinine.
Model 1: crude.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, BMI, smoking, SBP, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, history of CVD, use of ACEi/ARB, log SCr, and log ACR.

Predictive value of copeptin

The additional value of copeptin for risk prediction of cardiovascular and all-cause mortality was assessed in terms of discrimination (Harrell's C-statistic), NRI, and IDI (Table 4). Harrell's C statistics for models 2 and 3 without copeptin predicting cardiovascular mortality were 0.75 (0.71-0.78) and 0.80 (0.77-0.84) respectively. Harrell's C statistics for models 2 and 3 without copeptin predicting all-cause mortality were 0.77 (0.75-0.80) and 0.79 (0.77-0.82) respectively. Harrell's C statistics in Table 4 show that the more confounders we adjusted for, the better the model predicted cardiovascular and all-cause mortality. The Grønessby and Borgan *P*-values in Table 4 indicate that predicted probabilities correspond well with observed risks (except for model 1 predicting all-cause mortality), so the models were well calibrated. The IDI and NRI values for model 2 predicting cardiovascular mortality were 2.68% and 16.93% respectively, indicating that copeptin had additional value on top of age and gender for risk prediction of cardiovascular mortality. However, in the fully adjusted models, the IDI and NRI values appeared <2%, indicating that copeptin did not substantially improve risk prediction for cardiovascular and all-cause mortality beyond currently used clinical markers.

Table 4. Additional value of baseline log_2 copeptin concentrations in risk prediction compared with established cardiovascular risk markers.

	Harrell's C (95% CI)	IDI (%) (95% CI)	NRI (%) (95% CI)	Grønnessby and Borgan
CV Mortality				
Model 1	0.63 (0.59, 0.68)	NA	NA	0.10
Model 2	0.76 (0.72, 0.80)	2.68 (1.43, 3.93)	16.93 (6.53, 27.33)	0.98
Model 3	0.81 (0.77, 0.84)	0.14 (-0.27, 0.55)	1.82 (-4.11, 7.76)	0.15
All-Cause Mortality				
Model 1	0.63 (0.59, 0.66)	NA	NA	0.01
Model 2	0.78 (0.75, 0.80)	2.47 (1.45, 3.50)	2.14 (-2.99, 7.27)	0.84
Model 3	0.80 (0.77, 0.82)	0.77 (0.17, 1.37)	0.55 (-3.36, 4.46)	0.53

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; HDL, high-density lipoprotein; NA, not applicable; SCr, serum creatinine.
Model 1: crude.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, BMI, smoking, SBP, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, history of CVD, use of ACEi/ARB, log SCr, and log ACR.

Discussion

In this prospective cohort of 1,195 patients with type 2 diabetes treated in primary care, we found copeptin to be associated with cardiovascular and all-cause mortality. After adjustment for established cardiovascular risk factors, we observed a trend between copeptin and cardiovascular mortality, while the association of baseline plasma copeptin with all-cause mortality remained significant. Our findings are of particular interest since the AVP system is a potentially modifiable system through pharmacological and non-pharmacological interventions and could provide a possible target for treatment and prevention of cardiovascular events and mortality in type 2 diabetes.

Several studies have reported that plasma vasopressin levels are elevated in animals and patients with diabetes mellitus (4-7). Vasopressin promotes water reabsorption through stimulation of V_2 receptors, and it is suggested that increased levels of vasopressin limit glucose-induced water loss in patients with diabetes (23).

However, increased levels of vasopressin may have long-term deleterious renal and cardiovascular effects. In experimental animal studies, as well as in humans, it is shown that vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus (24,25). This notion is supported by the renal protective effects of vasopressin inhibition by drinking water or chronic treatment with a V_2 receptor antagonist in rats with renal failure (26,27) and diabetes (28).

High concentrations of plasma AVP are known to preferably stimulate V_{1a} receptors (8), that results in (coronary) vasoconstriction (29), which increases afterload, ventricular stress, and cardiac hypertrophy (8,30,31). Several studies have reported that copeptin, a surrogate for vasopressin, is associated with cardiovascular events and mortality in patients with cardiovascular diseases (i.e., acute myocardial infarction, heart failure, and stroke) (3). A recent study of Fenske et al. (19) showed that copeptin is associated with cardiovascular events, sudden death, and all-cause mortality in patients with type 2 diabetes and ESRD.

Stimulation of V_3 (V_{1b}) receptors through AVP results in the release of ACTH, which stimulates cortisol release from the adrenal gland (3). AVP-induced ACTH release, in contrast to corticotropin-releasing hormone (CRH) induced ACTH release, is reported to be less sensitive to feedback inhibition by glucocorticoids than that of CRH (32), which might worsen multiple aspects of the metabolic syndrome.

Median copeptin concentration of this study group was 5.4 pmol/L [range: 0.9-85.7 pmol/L], which is higher than the median copeptin level of 4.2 pmol/L [range: 1-13.8 pmol/L] measured in healthy subjects (33). In line with previous studies, we found baseline plasma copeptin to be associated with renal function and albuminuria (34,35).

In addition, we found that baseline copeptin concentration was associated with cardiovascular and all-cause mortality. However, we found no differences in the number of deaths, cardiovascular deaths or follow-up time between male and female subjects. This lack of difference is consistent with the literature on cardiovascular risk in diabetes, which indicates that women with diabetes have a higher relative risk for cardiovascular events compared to men with diabetes (36,37). Furthermore, copeptin values are consistently shown to be higher in males than in females, even in healthy subjects (12,19). Therefore, risk categories based on copeptin level or reference values for copeptin concentrations should be gender-specific.

During the past years, several studies have shown that copeptin measurement has diagnostic and prognostic value in patients with acute cardiovascular diseases (3). Since copeptin was found to be associated with cardiovascular and all-cause mortality in patients with type 2 diabetes, we investigated whether copeptin had additional value for risk prediction of cardiovascular and all-cause mortality. In this study

population, copeptin did not substantially improve risk prediction for cardiovascular and all-cause mortality beyond currently used clinical markers. However, copeptin was found to be associated with several cardiovascular risk factors (i.e., BMI, HbA_{1c}, systolic blood pressure, SCr, and ACR), and copeptin substantially improved risk prediction for cardiovascular mortality beyond age and gender. Thus, copeptin might be a unified marker for these known causes of CVD and might be useful to identify patients who would benefit for intensification of therapy (38).

We acknowledge that this study has several limitations. First, given the observational nature of this study, it is impossible to draw a definite conclusion about the causality of the association of copeptin with cardiovascular and all-cause mortality. Second, selection bias may have occurred because patients whose copeptin had not been measured were excluded from statistical analysis. However, in additional Cox regression analyses, we found no significant association of the presence or absence of a copeptin measurement with cardiovascular and all-cause mortality. Third, blood samples were taken without restriction on food or water intake, which could have influenced plasma osmolarity and consequently copeptin concentration. Furthermore, measured plasma osmolarity, data required to calculate plasma osmolarity, and data on the use of diuretics were not available in this study group, which is a limitation of the present study since plasma osmolality and plasma volume are determinants of AVP secretion. In addition, no data on plasma albumin levels and total plasma protein were available in the present study. It could have been interesting to include these measures since plasma albumin and protein might influence plasma osmolality and subsequently levels of AVP. Finally, the number of cardiovascular deaths in this study population is relatively small, which limits the number of covariates used in Cox regression analyses. It has been suggested that for each variable included in the model at least 10 events are required (20). With inclusion of 13 variables in the final Cox regression models, we are approaching the maximum number of variables allowed by the number of 148 cardiovascular deaths.

Strengths of this study are that this is the first study to investigate the association of copeptin with cardiovascular and all-cause mortality in patients with type 2 diabetes. In addition, this study included a relatively large observational cohort of patients with type 2 diabetes with a relatively long follow-up period (i.e., 10 years) and a reasonable number of events (i.e., all-cause mortality), therefore we could prospectively investigate the association of baseline plasma copeptin levels with cardiovascular and all-cause mortality.

In conclusion, in this cohort of patients with type 2 diabetes, plasma copeptin, a surrogate marker for vasopressin, was associated with cardiovascular and all-cause

mortality. These findings suggest that vasopressin may play a role in cardiovascular complications of type 2 diabetes and that interventions towards lowering vasopressin e.g. by limiting sodium intake, improving glycemic control, and improving/preventing nephropathy-associated albuminuria may be beneficial for the prevention of cardiovascular complications in type 2 diabetes. Water supplementation in patients without low serum albumin, edema, and not at risk for hyponatremia, would also be a possibility to lower AVP secretion. Furthermore, copeptin might be a unified marker for known causes of cardiovascular diseases and might be useful to identify patients who would benefit for intensification of therapy (38).

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Supplementary Table S1. Baseline patient characteristics of patients with and without copeptin measurement.

	Patients with copeptin measurement	Patients without copeptin measurement	*P*-value
Demographics			
Male gender (n, %)	558 (44.4)	192 (44.4)	0.9
Age (years)	67 ± 12	67 ± 12	0.9
Smoking (n, %)	215 (17.1)	85 (19.7)	0.3
History of CVD (n, %)	436 (34.7)	157 (36.3)	0.5
Body composition			
BMI (kg/m^2)	29.2 ± 4.8	29.0 ± 4.8	0.4
Blood pressure			
Systolic blood pressure (mmHg)	152 ± 24	150 ± 24	0.2
Diastolic blood pressure (mmHg)	84 ± 10	83 ± 10	0.5
Use of ACEi/ARB (n, %)	322 (25.6)	113 (26.2)	0.7
Lipids			
Total cholesterol (mmol/L)	5.6 ± 1.1	5.5 ± 1.1	0.2
HDL cholesterol (mmol/L)	1.2 ± 0.4	1.2 ± 0.9	0.2
Triglycerides (mmol/L)	2.1 [1.5-3.1]	2.1 [1.4-3.1]	0.6
Cholesterol-HDL ratio	5.0 ± 1.5	5.1 ± 1.7	0.3
Use of lipid lowering drugs (n, %)	186 (14.8)	82 (19.0)	0.1
Glucose homeostasis			
Duration diabetes (years)	4.0 [2.0-9.0]	4.0 [2.0-8.0]	0.2
HbA_{1c} (%)	7.0 [6.3-8.1]	6.9 [6.2-8.0]	0.1
HbA_{1c} (mmol/mol)	53 [45-65]	52 [44-64]	0.1
Renal function			
ACR (mg/mmol)	2.0 [0.9-7.1]	2.0 [0.9-8.1]	0.8
Serum creatinine (µmol/L)	95 ± 22	98 ± 23	0.04
eGFR (mL/min/1.73m^2)	64 ± 16	63 ± 16	0.05

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein.

Supplementary Table S2. Associations of baseline log_2 copeptin concentrations with cardiovascular and all-cause mortality in Cox regression analyses with time-dependent covariates.

	Cardiovascular Mortality		All-Cause Mortality	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Model 1	1.73 (1.48-2.03)	<0.001	1.57 (1.42-1.74)	<0.001
Model 2	1.60 (1.37-1.87)	<0.001	1.44 (1.30-1.59)	<0.001
Model 3	1.21 (1.03-1.42)	0.02	1.23 (1.10-1.36)	<0.001

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; HDL, high-density lipoprotein; SBP, systolic blood pressure; SCr, serum creatinine.
Model 1: crude.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, BMI (baseline), smoking (baseline), SBP, cholesterol-HDL ratio, duration of diabetes, HbA_{1c}, history of CVD (baseline), use of ACEi/ARB (baseline), log SCr, and log ACR.